Effects of dietary energy source and level and injection of tilmicosin phosphate on immune function in lipopolysaccharide-challenged beef steers

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Effects of dietary energy source and level and injection of tilmicosin phosphate on immune function in lipopolysaccharide-challenged beef steers[1,2]

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ABSTRACT: Twenty-four Angus × Hereford crossbred steers (247 kg BW; SE = 2.4 kg) were used in a completely random design to evaluate the effect of energy source and level with or without antibiotic administration on measures of immune function. Steers were fed 1 of 3 dietary treatments: a 70% concentrate diet ad libitum (70AL), a 30% concentrate diet ad libitum (30AL), and a 70% concentrate diet offered in an amount calculated to provide NE_g intake equal to the 30AL treatment (70RES). Half the steers in each dietary treatment received a s.c. injection of tilmicosin phosphate (ANTI; 1 mL/30 kg of BW); the other half received an equal volume of saline s.c. (SAL). Steers were offered the treatment diets for 28 d before and were administered the ANTI or SAL injections 2 d before indwelling catheters were placed in the jugular vein and 2.0 μg/kg of BW of *Escherichia coli* lipopolysaccharide (LPS) was administered i.v. Blood serum was collected at 30-min intervals from −2 to 6 h and at 8, 12, 24, 48, and 72 h relative to the LPS challenge. Increased energy intake (70AL) increased ($P \leq 0.04$) DMI, ADG, and rectal temperature (RT) after the challenge compared with the 70RES treatment. The 30AL treatment increased the maximum concentrations and area under the response curve of the proinflammatory cytokines (PIC) interferon-γ, tumor necrosis factor-α, and IL-6 ($P \leq 0.05$) compared with the average of the 70AL and 70RES treatments. Decreased energy intake (70RES vs. 70AL) increased IL-6 ($P \leq 0.003$) but did not significantly increase interferon-γ and tumor necrosis factor-α ($P \geq 0.14$) after LPS administration. Tilmicosin administration decreased the time to attain maximal RT ($P = 0.01$) by 1 h without altering the peak RT ($P = 0.85$), and tilmicosin interacted with energy intake to increase prechallenge PIC in 70RES vs. 70AL ($P \leq 0.05$). Results indicate that increased PIC response, presumably resulting from a combination of decreased energy intake and from direct effects of roughage, may be a mode of action for the slight decrease in morbidity that often occurs when newly received, stressed calves are fed roughage-based receiving diets. Tilmicosin phosphate might have immunomodulatory capacity beyond its direct effects on pathogenic bacteria, and these effects could interact with dietary energy intake in cattle.

Key words: beef cattle, energy, immune function, tilmicosin phosphate

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INTRODUCTION

Bovine respiratory disease (**BRD**) is the predominant health issue faced by the cattle industry, particularly when lightweight, stressed calves are received into feedlots (Galyean et al., 1999; Duff and Galyean, 2007). Preconditioning decreases the incidence of BRD (Roeber et al., 2001), but unfavorable economic results

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[2]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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limit its use by producers (Duff and Galyean, 2007). Metaphylactic antibiotic administration is one of the most effective options for decreasing BRD morbidity in high-risk cattle (Guthrie et al., 2004); however, antibiotic use increases cost and induces bacterial resistance concerns (Callaway et al., 2004).

Nutrition is known to be a major factor influencing the response of cattle to stress and disease challenges (Carroll and Forsberg, 2007). Dietary energy concentration and intake have been shown to influence health (Lofgreen et al., 1975; Fluharty and Loerch, 1996; Rivera et al., 2005) and immune function (Whitney et al., 2006) of newly received, stressed feedlot cattle. Nonetheless, most studies investigating energy effects on health have confounded the effects of energy concentration and energy source, because modifying the grain:roughage ratio of receiving diets is the most common method of altering energy concentration. Data regarding the effects of dietary energy concentration on physiological measures of immune function in stressed cattle are needed (Rivera et al., 2005).

Potential interactions of dietary energy concentration or intake and metaphylactic antibiotic treatment have not been evaluated. Our objective was to determine the separate effects of dietary energy source and energy concentration, with or without administration of an antibiotic, on the acute-phase immune response of beef steers. Because of its successful application in previous research (Steiger et al., 1999; Waldron et al., 2003) and the consistency of the acute-phase response that would be expected, we used an *Escherichia coli* lipopolysaccharide (**LPS**) challenge as our model.

MATERIALS AND METHODS

All procedures involving live animals were reviewed and approved by the Texas Tech University Animal Care and Use Committee.

The experiment was conducted at the Texas Tech University Burnett Center Research Feedlot, east of New Deal, TX, during November and December 2006.

Animals

In September 2006, crossbred beef bulls and steers (n = 116; average arrival BW = 212 kg, SE = 1.2 kg) were purchased at sale barns in central Texas and transported to The Samuel Roberts Noble Foundation Inc., Red River Demonstration and Research Farm, west of Marietta, OK. On arrival, the cattle were castrated with an elastic band (Callicrate Smart Bander; No Bull Enterprises Inc., St. Francis, KS) and were dehorned [Barnes-type dehorning tool (Moore Maker Inc., Matador, TX)] as needed and were administered clostridial (Covexin 8; Schering-Plough Animal Health Corporation, Kenilworth, NJ), respiratory viral (Vista 5 SQ; Intervet Inc., Millsboro, DE) and bacterial vaccines (Pulmo-guard PH-M; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), anthelmintic (Ivomec Plus; Merial Ltd. Duluth, GA), and metaphylactic antibiotic (ceftiofur crystalline free acid; Excede, Pfizer Inc., New York, NY) according to label directions. Skin biopsy samples from the pinna were collected, analyzed for persistent infection with bovine viral diarrhea virus by immunohistochemistry, and were determined to be negative for all cattle used in the experiment (Cattle Stats LLC, Edmond, OK). Three weeks after initial processing, booster injections of the respiratory viral and bacterial vaccines were administered, and the steers were implanted (Synovex C; 100 mg of progesterone and 10 mg of estradiol; Fort Dodge Animal Health, Overland Park, KS). Ten days after revaccination, 24 steers (247 ± 2.4 kg of BW) were selected for use in the experiment and transported to the Burnett Center. Steers were weighed and assigned randomly to 1 of 6 treatments in a completely random design. They were housed individually in 3.1 m × 16.8 m, soil-surfaced pens for the initial 21 d and for the final 19 d of the experiment. Pens were equipped with concrete feed bunks and automatic water fountains. During the intervening 10 d, in which a LPS challenge was administered (described below), the steers were housed individually inside an enclosed barn in 0.8 m × 2.1 m stanchions. Stanchions were equipped with feeders suspended from load cells and metered water bowls to facilitate measurement of individual feed and water intake. The barn was ventilated by fans at one end of the building and vents at the other end and was illuminated continuously during the 10-d period. At 0800 h on each day of the experiment, steers were fed individually, and orts were collected and weighed to determine daily DMI.

Treatments

Treatments were arranged in a 2 × 3 factorial. One factor was a s.c. injection on d 26 with either 1 mL/30 kg of BW of Micotil (300 mg of tilmicosin phosphate/mL; Elanco Animal Health, Greenfield, IN; ANTI) or a s.c. injection of an equal volume of physiological saline (SAL). The other factor was diet: a 70% concentrate diet fed ad libitum (70AL) diet, a 30% concentrate diet fed ad libitum (30AL), or the 70% concentrate diet fed in a quantity calculated to match the NE_g intake of the cattle receiving the 30AL treatment (70RES; NRC, 1996). The later treatment (70RES) restricted intake of the 70% concentrate diet, which allowed for evaluation of the effects of energy intake independent of energy source. Diet composition data are shown in Table 1.

Challenge and Blood Sampling

Blood samples were collected by jugular venipuncture on d 0, 7, 14, 21, 27, 28, 29, 30, 31, 35, 43, and 49. Serum was collected from each sample after a 1-h clotting time after collection at room temperature and centrifugation for 20 min at 2,000 × *g* and 4°C. Serum was stored at −80°C until assayed. On d 27, the jugular vein of each steer was catheterized with a 14 ga × 140 mm Abbocath-T (Hospira Inc., Lake Front, IL) catheter fitted with a 1-m extension (1.4 mm o.d.

polytetrafluoroethylene tubing; Tygon, Saint-Gobain Performance Plastics Corp., Paris, France). On d 28, steers were challenged i.v. with 2.0 μg/kg of BW of LPS from *E. coli* 0111:B4 (Sigma-Aldrich, St. Louis, MO) in a bolus of 2.4 to 2.8 mL of physiological saline. Blood samples were collected through the jugular catheter at 30-min intervals from −2 to 6 h and again at 8, 12, 24, 48, and 72 h relative to LPS challenge (**RTC**). At each sampling time, catheters were aspirated, and 5 mL of blood was drawn and discarded. Subsequently, approximately 9 mL of blood was collected into a 16 mm × 160 mm plastic serum separation tube (Monovette, Sarstedt Inc., Newton, NC). Catheters and extensions were then flushed with 5 mL of saline followed by 2.5 mL of heparinized saline (20 IU of heparin/mL). Serum was collected and stored as described above. A timeline of events during the experiment is shown in Table 2.

Immune and Endocrine Analyses

Serum cortisol concentration was determined by a commercial radioimmunoassay kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, intraassay CV was 10%) that has been used successfully in cattle (Merrill et al., 2007). Serum concentration of 4 cytokines was assayed using a custom-developed multiplex ELISA validated for bovine cytokines (SearchLight; Pierce Biotechnology Inc., Rockford, IL). The multiplex ELISA assayed the proinflammatory cytokines (**PIC**; cytokine abbreviation followed by intra- and interassay CV, respectively) tumor necrosis factor-α (**TNF-α**; 6.5%, 16%), interferon-γ (**IFN-γ**; 10.4%, 16.6%), and IL-6 (6.5%, 17.7%) and the antiinflammatory cytokine IL-4 (5.3%, 19.9%). Concentration of the acute-phase protein serum amyloid-A (**SAA**) was determined using a commercially available ELISA kit (BioSource International Inc., Camarillo, CA; intra- and interassay CV were 4.1% and 12.1%, respectively).

Humoral Immune Response

On d 31, the steers were given an injection of ovalbumin as a novel antigen to measure effects of treatments on humoral immune response. The challenge was administered in a manner similar to that described by Rivera et al. (2002). Steers were injected (s.c.) with 4 mL of an emulsion containing 4 mg of albumin from chicken egg white in a 1:1 ratio of Freund's incomplete adjuvant and phosphate-buffered saline (Sigma-Aldrich) on d 31, and again on d 43. Blood samples were collected into tubes containing EDTA on d 31, 35, 43, and 49. Samples were allowed to stand at ambient temperature for 1 h after collection. Plasma was then collected from the samples after a 20-min centrifugation at 2,000 × *g* and 4°C. Plasma samples were stored at −80°C until assayed. Immunoglobulin G (**IgG**) specific to ovalbumin was measured by colorimetric (alkaline phosphatase conjugated anti-bovine IgG with *p*-nitrophenyl phosphate substrate) ELISA as described by Rivera et al. (2002) with 2 modifications. First, to remove possible cross-reactive antibodies, plasma samples were incubated for 24 h at 4°C with a solution containing 0.5 mg of bovine albumin, similar to the procedure described by Ameiss et al. (2004). The second modification was to initially dilute plasma samples from d 31, 35, 43, and 49 by factors of 100, 200, 800, and 3,200, respectively. Three hundred microliters of these initial dilutions was added to the top row of a 96-well plate in duplicate, and 150 μL of buffer was added to the remaining rows. Serial dilutions were accomplished by transferring 150 μL from the top row to the subsequent row down the plate and mixing. This process was repeated for each row. After mixing, 150 μL was removed from the last row and discarded. A column was reserved

Table 1. Description of experimental diets (% of DM)

Item	70% concentrate	30% concentrate
Ingredient		
Steam-flaked corn	52.25	13.00
Cottonseed hulls	15.00	35.00
Ground alfalfa hay	14.98	34.99
Cottonseed meal	8.66	8.01
Molasses	4.00	4.00
Supplement[1]	2.50	2.50
Fat (yellow grease)	2.00	2.01
Urea	0.61	0.49
Analyzed composition[2]		
CP, %	13.38	12.80
ADF, %	19.77	36.77
Fat, %	4.55	4.27
Ca, %	0.66	0.69
P, %	0.28	0.22
K, %	0.96	1.36
Calculated composition[3]		
Degradable intake protein	8.84	9.92
NE_m, Mcal/kg	1.83	1.32
NE_g, Mcal/kg	1.19	0.75
Effective NDF, %	23.0	47.5
Mg, %	0.28	0.33
S, %	0.23	0.25
Co, mg/kg	0.61	0.51
Cu, mg/kg	21.4	25.5
Fe, mg/kg	155.0	213.4
I, mg/kg	0.60	0.60
Mn, mg/kg	76.07	109.28
Se, mg/kg	0.23	0.35
Zn, mg/kg	91.0	101.1
Na, %	0.16	0.19
Cl, %	0.43	0.41
DCAD, mEq/kg	50.6	146.4

[1]Supplement contained (DM basis): 23.368% cottonseed meal; 0.500% Endox (antioxidant; Kemin Industries, Des Moines, IA); 42.105% limestone; 1.036% dicalcium phosphate; 8.000% potassium chloride; 3.559% magnesium oxide; 6.667% ammonium sulfate; 12.000% salt; 0.0017% cobalt carbonate; 0.157% copper sulfate; 0.133% iron sulfate; 0.0025% ethylenediamine dihydroiodide; 0.267% manganese oxide; 0.100% selenium premix (0.2% Se); 0.845% zinc sulfate; 0.0079% vitamin A (1,000,000 IU/g); 0.126% vitamin E (500 IU/g); 0.675% Rumensin (176.4 mg/kg; Elanco Animal Health, Greenfield, IN); and 0.45% Tylan (88.2 mg/kg; Elanco Animal Health). Concentration values in parentheses are expressed on a 90% DM basis.

[2]Samples collected twice weekly were composited and analyzed by a commercial laboratory.

[3]Nutrient composition calculated from tabular values in NRC (1996). DCAD = dietary cation-anion difference, mEq [(Na + K) − (Cl + S)]/100 g of DM.

Table 2. Description of events and timeline

Day	Event
−4	Steers delivered to Texas Tech University Burnett Center
0	Begin feeding treatment diets in soil-surfaced pens, blood sample, BW
7	Blood sample, BW
14	Blood sample, BW
21	Blood sample, BW, move to stanchion barn
26	Antibiotic administration
27	Blood sample, BW, jugular catheterization, install rectal temperature (RT) probe
28	Lipopolysaccharide challenge, intensive blood sampling
29	Blood sample
30	Blood sample
31	Blood sample, BW, administer ovalbumin, return to soil-surfaced pens, remove RT probe
35	Blood sample, BW
43	Blood sample, BW, booster ovalbumin
49	Blood sample, BW

for nonspecific binding for each day, which resulted in 3 columns used for each day; therefore, all 4 d for each animal were analyzed on 1 plate. Absorbance at 405 nm was determined using a plate reader (Benchmark, Bio-Rad Laboratories, Hercules, CA). The intraassay CV was 6.2%.

BW and Temperature

Concurrent with weekly blood sampling, steers were weighed individually on calibrated scales. The individual animal scale was a hydraulic squeeze chute (Ultraline, Cummings Sales Inc., Garden City, KS) set on 4 load cells (Rice Lake Weighing Systems, Rice Lake, WI) that was calibrated with certified weights (453.59 kg, Texas Department of Agriculture, Austin) within 24 h of use. From d 27 to 31, rectal temperature (**RT**) of each steer was measured at 1-min intervals with an automatic RT recording device (Reuter et al., 2007). Temperature probes were immersed in a common container of water for 1 h before attaching them to the animals, and RT collected from the animals was corrected for initial differences among probes.

Statistical Analyses

Final BW of cattle on the 70RES treatment was adjusted for treatment-imposed differences in gut fill by subtracting the average daily DMI of 70RES steers from the average daily DMI of 70AL steers and adding that value back to the observed final BW of 70RES steers. Adjusted final BW was used to calculate ADG. Body weight, DMI, and other nonrepeated measures data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC) as a completely random design with a 2 × 3 factorial arrangement of treatments. Dietary and injection treatments, as well as the interaction, were fixed effects in the model. Response to LPS challenge over time (repeated-measures response; **RMR**) for immune function measures and 30-min averages of RT was determined by repeated-measures ANOVA with the PROC MIXED procedure of SAS using an autoregressive covariance structure. In addition, prechallenge concentration (average of −2- to 0-h samples), maximum concentration after challenge (maximum observed value from 0- to 72-h samples), time that the maximum concentration was reached (time period associated with maximum concentration), and the area under the response curve (**AUC**; calculated by trapezoidal summation) were determined for each variable and analyzed using PROC MIXED as for other nonrepeated measures data. Individual animal was the experimental unit in all analyses. Orthogonal contrasts were used to evaluate the effects of energy source [30AL vs. (70AL + 70RES)/2], energy level (70AL vs. 70RES), injection treatments, and the 2-way interactions. In the event of significant interaction contrasts, least squares means were separated using the PDIFF option of PROC MIXED. Significance was declared at $\alpha \leq 0.05$, and trends were declared at $\alpha \leq 0.10$. Pearson correlation coefficients were calculated among PIC, SAA, RT, and ADG using PROC CORR of SAS.

Concentration of IgG specific to ovalbumin was determined by a LOGIT regression in SAS using all dilutions for a sample to estimate the specific dilution at which 50% relative binding would have occurred. Briefly, within an animal and day combination, absorbance at each dilution was divided by the maximum absorbance (the first dilution) to yield the percentage of relative binding for each dilution. Percentage of relative binding was divided by 1 minus the relative fractional binding. The natural logarithms of these values were regressed against the natural logarithms of the respective dilutions. The simple linear regression equation was set equal to the natural logarithm of 50% binding and algebraically rearranged to estimate the natural logarithm of the dilution at which 50% binding would have occurred. This estimate was then converted into actual dilutions by raising the base of the natural logarithm (e) to the power of the estimate. The base-2 logarithm of these estimates was then analyzed to decrease the potential for heterogeneity of variance. Backtrans-

formed means are reported in the table and text. Because this method uses several estimates of binding for each sample, this method is preferred over the traditional method of selecting only a single dilution at which to compare treatments (J. Vizcarra, Texas Tech University, Lubbock, personal communication). The resulting estimates of IgG concentration were analyzed by repeated measures as described above.

Rectal temperature devices malfunctioned in 5 steers (1, 2, and 2 steers in the 70AL × SAL, 30AL × ANTI, and 70RES × ANTI treatments, respectively); therefore, useable RT data were collected from 19 steers. The jugular catheter malfunctioned in 1 steer in the 30AL × SAL treatment at 8 and 12 h RTC, thereby preventing serum collection. Two steers in the 30AL × ANTI treatment died (described in a subsequent section), 1 at 9 h RTC and the other at 23 h RTC, thereby decreasing sample size in that treatment to 2 for the remainder of the experiment. All data from the 2 steers that died were removed from the analysis of SAA, because samples were not available for the time period when the bulk of the SAA response occurred in the other steers.

RESULTS AND DISCUSSION

Intake and Growth

By design, DMI was greater by 70AL steers than by 70RES steers (Table 3; $P < 0.001$) and greater by steers receiving the 30AL than by steers fed the 70% concentrate diets ($P < 0.001$). As expected, greater NE_g intake by 70AL steers resulted in increased ADG and final BW compared with 70RES steers ($P < 0.02$). Berry et al. (2004b) fed diets that differed in energy and starch concentration to stressed calves for 42 d and observed no effect of diet on ADG. Similar to our results, DMI was greater with low-energy diets than with high-energy diets; however, the range of energy concentrations in diets fed by Berry et al. (2004b) was smaller than ours. Some caution is warranted when interpreting DMI in our study, because the difference in DMI between high-concentrate and high-roughage diets is largely a result of the mathematical structure of the orthogonal contrasts we tested (e.g., comparing the average of 70AL and 70RES vs. 30AL). The focus of the current study, however, was not measurement of responses in DMI, ADG, or morbidity. Readers interested in these variables as affected by dietary roughage concentration are referred to Rivera et al. (2005).

Temperature

Rectal temperatures averaged over 30-min intervals are shown in Figure 1. Temperature increased for 2 to 3 h RTC and then returned to baseline by 5 h RTC. The febrile response to LPS was relatively transient, indicating the need to measure temperature frequently with this type of experimental model (Davis et al., 2003). Although the direct effects of fever on performance of cattle are unknown, RT is the most common objective measurement associated with assessment of morbidity in commercial cattle production in the United States. Therefore, the effects of our treatments on RT and how RT is related to the other indicators of immune function measured in the present study would likely be of practical interest.

No effects ($P > 0.16$; Table 4) of antibiotic injection or dietary treatments were noted for prechallenge RT

Table 3. Effect of energy source and level with or without antibiotic injection on growth performance by steers during a 50-d period that included a lipopolysaccharide challenge[1]

	Saline			Tilmicosin				
Item	70AL	30AL	70RES	70AL	30AL	70RES	SE[2]	Contrasts[3]
Steers, n	4	4	4	4	2	4	—	—
Initial BW, kg	236	246	250	241	244	245	6.0	—
Final BW,[4] kg	286	275	272	279	286	267	9.0	—
ADG,[4] kg/d	1.03	0.59	0.44	0.77	0.81	0.44	0.08	2, 4
DMI, kg/d	13.8	15.0	9.4	12.9	13.7	9.2	0.59	1, 2
NE_g intake,[5] Mcal/d	4.5	2.5	2.1	4.1	2.2	2.1	0.23	1, 2

[1]Treatments were arranged in a 3 × 2 factorial. Dietary treatments were a 70% concentrate diet fed ad libitum (70AL), a 30% concentrate diet fed ad libitum (30AL), and a 70% concentrate diet restricted to equal calculated NE_g intake of 30AL treatment (70RES). Antibiotic treatments were either saline or tilmicosin phosphate (Micotil, 300 mg of tilmicosin/mL; Elanco Animal Health, Greenfield, IN) at 1 mL/30 kg of BW injected on d 26.

[2]Largest SE of the simple-effect least squares means.

[3]Indicates that the respective contrast was significant ($P < 0.05$); 1 = energy source: 30AL vs. (70AL + 70RES/2); 2 = energy level: 70AL vs. 70RES; 3 = antibiotic: tilmicosin vs. saline; 4 = interaction of energy source and antibiotic; and 5 = interaction of energy level and antibiotic.

[4]Final BW and ADG data for the 70RES treatment were adjusted for differences in fill imposed by restricted DMI by calculating the difference in average daily DMI between 70AL and 70RES treatments and adding that value to 70RES final BW. Adjusted final BW was used to calculate adjusted ADG.

[5]NE_g intake was determined by multiplying DMI by NE_g concentration from tabular values in NRC (1996).

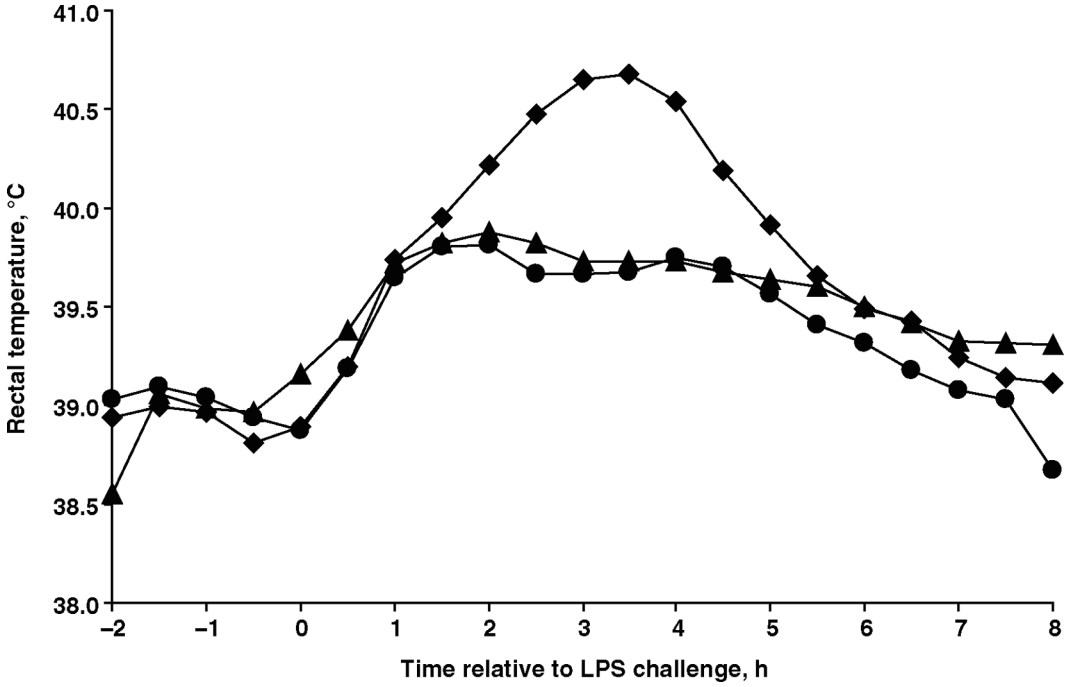

Figure 1. Least squares means of rectal temperature in steers challenged with lipopolysaccharide (LPS; largest main effect × sampling time SE = 0.47). Dietary treatments were a 70% concentrate diet fed ad libitum (♦, n = 7), a 30% concentrate diet fed ad libitum (▲, n = 6), and a 70% concentrate diet fed in a quantity restricted to equal the NE_g intake of the 30% concentrate treatment (●, n = 6). Increased energy intake increased the difference in prechallenge and peak rectal temperature ($P = 0.04$).

or for maximum RT after LPS challenge. Nonetheless, greater energy intake (70AL vs. 70RES; contrast 2, Table 4) increased the difference between prechallenge and maximum RT ($P = 0.04$). Energy intake has been previously associated with increased febrile response to an immune challenge in cattle (Perkins et al., 2002; Whitney et al., 2006); however, energy intake did not affect RT in steers that were simply stressed by transportation and not experimentally challenged (Holt et al., 2003). Perhaps these conflicting results indicate that dietary energy level interacts with type and intensity of stress to affect RT.

Administration of tilmicosin phosphate 2 d before the challenge decreased ($P = 0.01$; Table 4; Figure 2) the elapsed time between LPS challenge and maximum RT by 1 h. This result was somewhat unexpected, because these steers were not challenged with live pathogenic organisms. The antibiotic could have influenced subacute levels of pathogens in these steers. Alternatively, it might have directly altered basal cytokine production, thereby influencing the febrile response. Nonetheless, no main effect of injection with tilmicosin phosphate was observed in prechallenge RT or immune functions measured in this experiment. Fajt et al. (2004) observed that tilmicosin decreased RT to a greater extent than danofloxacin, and both antibiotics decreased temperature compared with saline, when injected into heifers 21 h after they were challenged with *Mannheimia hemolytica*. Because that challenge involved live organisms, the response could have been the result of a direct action of the antibiotic in decreasing the intensity of the infection. Fajt et al. (2003) observed no effect of antibiotic on neutrophil function in cattle; therefore, potential cytokine production from neutrophils would not likely be altered by antibiotic administration. Elsasser et al. (2007) suggested that endotoxin released into circulation when antibiotics lyse pathogens in vivo could be a source of additional endotoxin, which could indirectly change the cytokine and associated RT response. Waldron et al. (2003) and Reuter (2007) indicated that the febrile response in LPS-challenged cattle may be independent of the LPS dose, or that it is maximized at LPS doses much lower than those used in our experiment. Additional research is needed to measure the direct effect of tilmicosin phosphate on cytokine production in immunologically challenged animals. If this effect is beneficial and could be mimicked by other nonantibiotic drugs, it might provide a means to enhance immune function in receiving cattle, while avoiding the potential of bacterial resistance associated with antibiotic use.

Mortality

Two animals died after the LPS challenge. Both animals were assigned to the 30AL treatment and received tilmicosin phosphate. Despite emergency supportive therapy including epinephrine and nonsteroidal anti-inflammatory injections, 1 animal died 9 h after LPS challenge and the other died 23 h after LPS challenge.

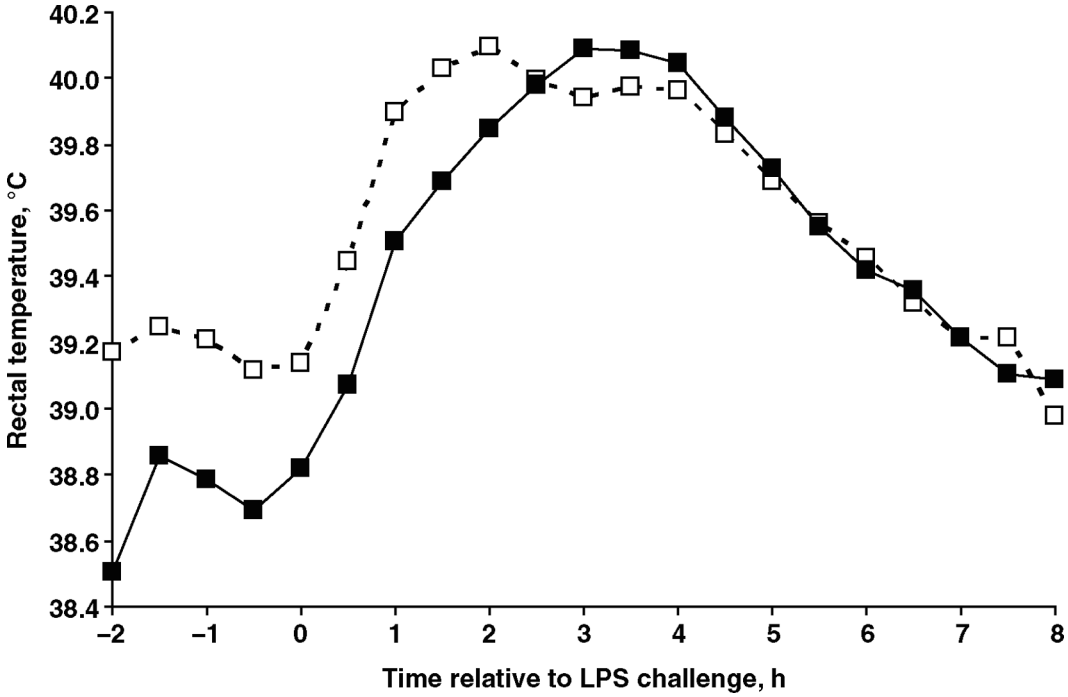

Figure 2. Least squares means of rectal temperature in steers challenged with lipopolysaccharide (LPS; largest main effect × sampling time SE = 0.40). Treatments were injection of 30 mg of tilmicosin phosphate/kg of BW (□, n = 8) or saline (■, n = 11). Injection of tilmicosin phosphate decreased the elapsed time from LPS administration to the rectal temperature peak ($P = 0.01$).

Based on necropsy results, the animal that died at 9 h had a consolidated lung abscess that apparently ruptured after LPS challenge. Necropsy results from the animal that died at 23 h indicated no chronic conditions, and cause of death was determined to be toxic shock. Mortality was not an expected outcome of the LPS challenge. The LPS dose used in this experiment (2.0 µg/kg of BW) has been used successfully by other

Table 4. Effect of energy source and level with or without antibiotic injection on rectal temperature of steers challenged with lipopolysaccharide (LPS)[1]

	Saline			Tilmicosin				
Item[2]	70AL	30AL	70RES	70AL	30AL	70RES	SE[3]	Contrasts[4]
Rectal temperature, °C								
Steers,[5] n	3	4	4	4	2	2	—	—
Prechallenge	38.9	38.6	38.7	38.8	39.3	39.4	0.64	—
Maximum	41.2	40.1	40.7	40.5	40.1	40.7	0.49	—
Change	2.2	1.5	1.3	1.7	0.8	1.1	0.45	2
Maximum time, min	207	275	236	213	113	92	51.8	3
AUC, 8 h	19,246	19,007	18,879	19,081	19,083	19,073	200	—

[1]Treatments were arranged in a 3 × 2 factorial. Dietary treatments were a 70% concentrate diet fed ad libitum (70AL), a 30% concentrate diet fed ad libitum (30AL), and a 70% concentrate diet restricted to equal calculated NE_g intake of 30AL treatment (70RES). Antibiotic treatments were either saline or tilmicosin phosphate (Micotil, 300 mg of tilmicosin/mL; Elanco Animal Health, Greenfield, IN) at 1 mL/30 kg of BW injected on d 26.

[2]The number of steers reflects those with sufficient data for inclusion in the analysis. Prechallenge = average of values from −2 to 0 h relative to LPS challenge; maximum = peak value observed from 0 to 8 h relative to LPS challenge; change = difference in maximum value and prechallenge value; maximum time = time at which maximum value was attained; AUC = area under the response curve by trapezoidal summation for 0 to 8 h relative to LPS challenge, arbitrary units.

[3]Largest SE of the simple-effect least squares means.

[4]Indicates the respective contrast was significant ($P < 0.05$); 1 = energy source: 30AL vs. (70AL + 70RES/2); 2 = energy level: 70AL vs. 70RES; 3 = antibiotic: tilmicosin vs. saline; 4 = interaction of energy source and antibiotic; and 5 = interaction of energy level and antibiotic.

[5]Five temperature recorders malfunctioned, preventing data collection. Two steers in the tilmicosin-30AL treatment died after the LPS challenge; however, data from these 2 steers are included in the time periods summarized in this table.

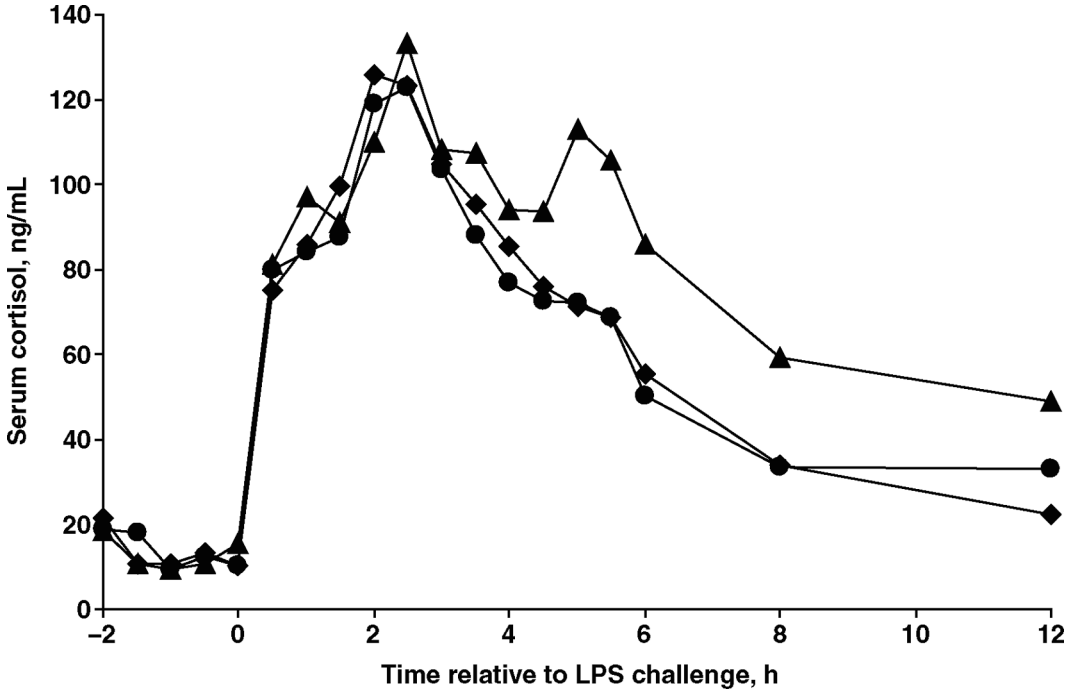

Figure 3. Least squares means of serum cortisol concentration in steers challenged with lipopolysaccharide (LPS; n = 6 to 8 steers/treatment; largest main effect × sampling time SE = 11.0). Dietary treatments were a 70% concentrate diet fed ad libitum (♦), a 30% concentrate diet fed ad libitum (▲), and a 70% concentrate diet fed in a quantity restricted to equal the NE_g intake of the 30% concentrate treatment (●). The 30% concentrate diet increased the elapsed time from LPS administration to the cortisol concentration peak ($P = 0.01$) and the overall cortisol response across time in a repeated-measures model ($P = 0.03$).

researchers (Steiger et al., 1999; Elsasser et al., 2005) and was used in a preliminary experiment in our own laboratory (Reuter, 2007) without incident. Nonetheless, several experimental conditions differed between the preliminary experiment described by Reuter (2007) and the current experiment, including age, BW, and body fat content of the steers. Perhaps some of these factors influence the intensity of the response to LPS. In future experiments with cattle similar to ours, lower doses of LPS would seem appropriate.

Cortisol

When cattle are stressed, cortisol is released to maintain homeostasis. In an immune challenge, cortisol can have an inhibitory feedback on PIC. Overall, the cortisol response to LPS (Figure 3) in the current study was intermediate in magnitude but similar in shape to those reported by Steiger et al. (1999) and Waldron et al. (2003). The high-roughage diet extended the time elapsed between the challenge and attainment of maximal cortisol concentration ($P = 0.01$; Table 5), without altering the maximal concentration ($P = 0.56$). This difference in time to reach peak cortisol concentration was caused by a biphasic response in cortisol in the 30AL treatment group, with an initial peak similar to the high-concentrate diet but also a secondary peak at 5 h RTC (Figure 3). This secondary peak was not observed in the high-concentrate diet. The high-roughage diet also increased the cortisol response across time in a repeated-measures model ($P = 0.03$). To our knowledge, no previous published research has documented the effects of energy source and level on the cortisol response of LPS-challenged cattle.

Cytokines and Acute-Phase Protein

Although PIC and acute-phase proteins are integral to the acute-phase immune response, relatively few studies in beef cattle have measured them. Maximum concentration, AUC, and RMR of IFN-γ and TNF-α were greater ($P < 0.05$; Table 5) with the 30AL treatment than with the average of the 70AL and 70RES treatments (Figures 4 and 5). Numerically, the PIC response in the 70RES treatment was intermediate to the response in the 70AL and 30AL treatments, indicating that decreased energy intake could be responsible for a portion of the effects observed. Maximum concentration, AUC, and RMR of IL-6 were decreased in 70AL compared with 70RES ($P \leq 0.003$; Table 5; Figure 6). This finding would seem to indicate that the source of dietary energy, as well as energy intake or energetic plane of nutrition, affects the PIC response to a LPS challenge. Berry et al. (2004a,b) observed a trend ($P = 0.11$) for low-starch diets to decrease morbidity independent of energy concentration. Although these researchers found no effects of dietary energy on acute-phase protein production, greater energy intake

decreased the percentage of calves morbid with *Pasteurella multocida* and *Hemophilus somnus*. No conclusions can be drawn as to the potential relationship of PIC concentration and subsequent morbidity from their study, because PIC were not measured.

The greater serum PIC response we observed with LPS challenge might allow cattle to respond more efficiently to disease pathogens encountered in the feedlot environment. Rivera et al. (2005) reported a slight decrease in percentage of cattle treated for BRD when newly received cattle were fed diets with greater roughage concentrations. This response might result, in part, from greater serum PIC associated with decreased energy intake, as well as a direct effect of replacing grain with roughage in the diet. Because LPS administration is an acute, artificial challenge compared with naturally acquired BRD complex, additional research is needed to test the hypothesis that a more intense PIC response is beneficial to the health of cattle in a commercial environment.

The 30AL diet decreased ($P = 0.05$; Table 5) prechallenge levels of IL-4 to near zero. Antiinflammatory cytokines generally inhibit PIC and promote humoral immunity (Elenkov et al., 2005; Carroll and Forsberg, 2007). This effect may underlie the greater PIC response observed with the 30AL diet.

Administration of tilmicosin phosphate tended ($P = 0.06$) to decrease the elapsed time from LPS challenge

Table 5. Effect of energy source and level with or without antibiotic injection on cortisol, cytokine, serum amyloid A, and humoral immune response of steers challenged with lipopolysaccharide (LPS)[1]

	Saline			Tilmicosin				
Item[2]	70AL	30AL	70RES	70AL	30AL	70RES	SE[3]	Contrasts[4]
Cortisol, ng/mL								
Prechallenge	13	14	14	14	12	13	1.2	—
Maximum	150	162	129	151	151	164	16.3	—
Maximum time, h	2.4	3.0	2.3	2.4	6.3	2.8	1.0	1
AUC, 12 h	2,385	2,671	2,127	2,053	2,353	2,171	238	—
IFN-γ, pg/mL								
Prechallenge	16	26	13	9	30	67	21	—
Maximum	443	3,832	1,013	52	3,806	2,134	1,542	1
Maximum time, h	9.1	4.6	21.6	32.4	4.4	4.1	9.9	5
AUC, 72 h	5,219	38,789	10,359	815	35,803	25,251	14,488	1
TNF-α, pg/mL								
Prechallenge	17	17	19	22	30	785	183	5
Maximum	4,100	11,174	6,568	6,289	17,108	8,695	3,296	1
Maximum time, h	1.4	1.4	1.6	1.1	1.3	2.5	0.43	—
AUC, 72 h	24,027	73,815	38,002	29,154	125,630	72,678	23,332	1
IL-4, pg/mL								
Prechallenge	21	1	20	38	1	43	16	1
Maximum	68	82	82	84	29	416	103	—
Maximum time, h	44	37	54	30	26	49	13.4	—
AUC, 72 h	681	129	677	1,295	61	2,851	957	—
IL-6, pg/mL								
Prechallenge	20	23	41	37	35	262	37	5
Maximum	8,148	15,982	13,829	4,675	14,116	12,655	1,957	1, 2
Maximum time, h	5.1	5.8	5.4	4.8	5.8	6.4	0.49	—
AUC, 72 h	90,104	172,232	142,434	57,218	139,069	142,622	18,664	1, 2
SAA, ng/mL								
Prechallenge	81	322	510	490	98	204	179	5
Maximum	1,571	2,022	2,797	1,576	1,268	1,306	683	—
Maximum time, h	42	56	35	21	48	33	15.4	—
AUC, 72 h	622	1,662	2,403	1,956	1,011	920	871	5
Ovalbumin response[5]	1,864	2,183	1,130	1,741	1,633	2,866	—	—

[1]Treatments were arranged in a 3 × 2 factorial. Dietary treatments were a 70% concentrate diet fed ad libitum (70AL), a 30% concentrate diet fed ad libitum (30AL), and a 70% concentrate diet restricted to equal calculated NE_g intake of 30AL treatment (70RES). Antibiotic treatments were either saline or tilmicosin phosphate (Micotil, 300 mg of tilmicosin/mL; Elanco Animal Health, Greenfield, IN) at 1 mL/30 kg of BW injected on d 26.

[2]TNF-α = tumor necrosis factor-α; IFN-γ = interferon-γ; SAA = serum amyloid A. Prechallenge = average of values from −2 to 0 h relative to LPS challenge; maximum = peak value observed from 0 to 8 h relative to LPS challenge; maximum time = time at which maximum value was attained; AUC = area under the response curve by trapezoidal summation for the time period indicated relative to LPS challenge, arbitrary units.

[3]Largest SE of the simple-effect least squares means; n = 4 steers per diet × antibiotic combination, except that n = 2 steers in the combination of 30AL and tilmicosin treatments for AUC of all variables, all SAA responses, and ovalbumin responses; and n = 3 steers in the combination of 30AL and saline treatments for AUC of all variables.

[4]Indicates the respective contrast was significant ($P < 0.05$); 1 = energy source: 30AL vs. (70AL + 70RES/2); 2 = energy level: 70AL vs. 70RES; 3 = antibiotic: tilmicosin vs. saline; 4 = interaction of energy source and antibiotic; and 5 = interaction of energy level and antibiotic.

[5]Estimated dilution at which 50% binding of antibody would occur, as determined from LOGIT regression analysis.

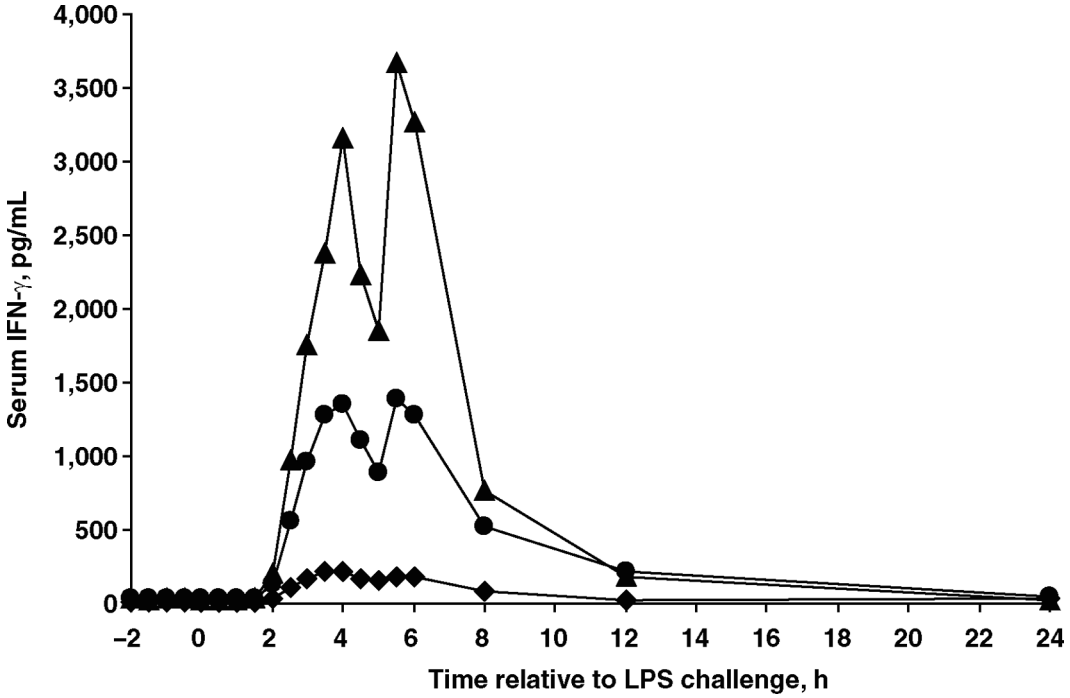

Figure 4. Least squares means of serum interferon-γ (IFN-γ) concentration in steers challenged with lipopolysaccharide (LPS; n = 6 to 8 steers/treatment; largest main effect × sampling time SE = 473). Dietary treatments were a 70% concentrate diet fed ad libitum (♦), a 30% concentrate diet fed ad libitum (▲), and a 70% concentrate diet fed in a quantity restricted to equal the NE_g intake of the 30% concentrate treatment (●). The 30% concentrate diet increased the overall IFN-γ response across time in a repeated-measures model ($P = 0.03$).

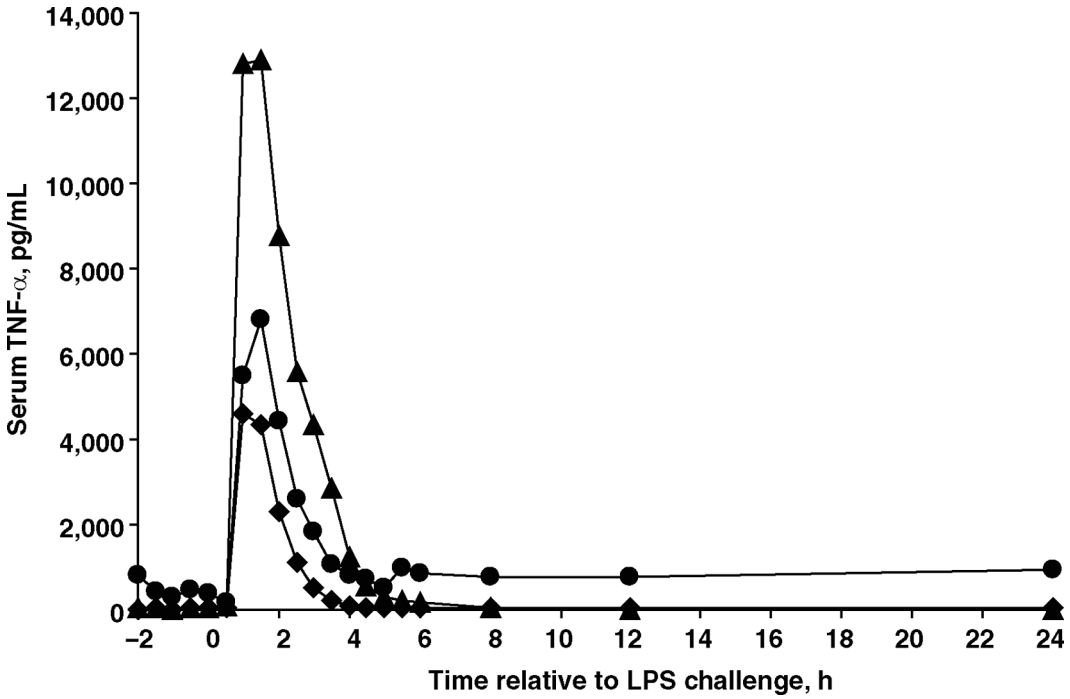

Figure 5. Least squares means of serum tumor necrosis factor-α (TNF-α) concentration in steers challenged with lipopolysaccharide (LPS; n = 6 to 8 steers/treatment; largest main effect × sampling time SE = 886). Dietary treatments were a 70% concentrate diet fed ad libitum (♦), a 30% concentrate diet fed ad libitum (▲), and a 70% concentrate diet fed in a quantity restricted to equal the NE_g intake of the 30% concentrate treatment (●). The 30% concentrate diet increased the overall TNF-α response across time in a repeated-measures model ($P = 0.02$).

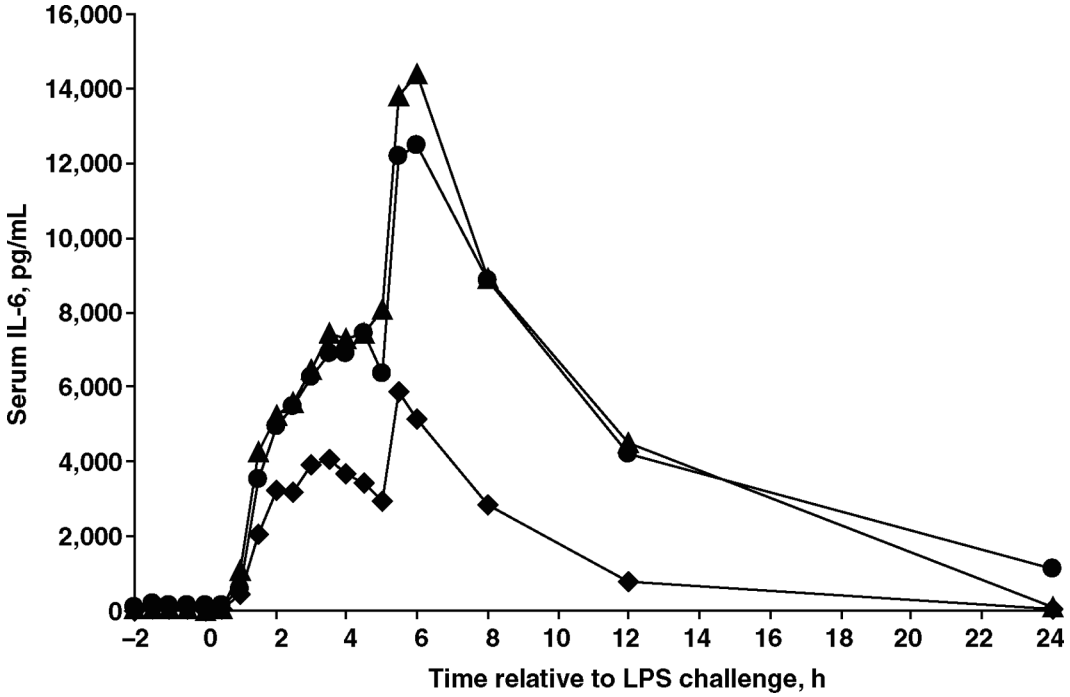

Figure 6. Least squares means of serum IL-6 concentration in steers challenged with lipopolysaccharide (LPS; n = 6 to 8 steers/treatment; largest main effect × sampling time SE = 771). Dietary treatments were a 70% concentrate diet fed ad libitum (♦), a 30% concentrate diet fed ad libitum (▲), and a 70% concentrate diet fed in a quantity restricted to equal the NE_g intake of the 30% concentrate treatment (●). Increased energy intake decreased the overall IL-6 response across time in a repeated-measures model ($P = 0.002$).

until the maximum SAA concentration was achieved (Table 5; Figure 7), but it did not ($P = 0.35$) alter the maximum concentration. In the 70RES treatment, tilmicosin decreased SAA AUC and RMR ($P < 0.04$; Table 5; Figure 7); however, tilmicosin injection had no effect in the 70AL treatment. Antibiotic administration decreased serum haptoglobin concentration in stressed steers (Wittum et al., 1996), but these results were obtained from nonchallenged animals in a commercial environment over a 65-d period. Carter et al. (2002) reported that SAA is involved in repairing cellular damage resulting from an infection or injury. Tilmicosin phosphate may have decreased this cellular damage in the 70RES treatment, thereby indirectly decreasing SAA. Alternatively, tilmicosin phosphate may directly mitigate increased SAA response in cattle fed energy-restricted diets. Tilmicosin tended ($P = 0.09$; Figure 8) to increase TNF-α RMR. Because SAA is stimulated by TNF-α (Alsemgeest et al., 1996), and TNF-α is involved in control of the febrile response (Johnson, 2002), additional TNF-α could cause the more intense SAA and febrile responses we observed.

Interactions and Humoral Response

Several interactions between dietary energy level and antibiotic injection were noted (Table 5). Prechallenge concentrations of some PIC were greater (TNF-α and IL-6; $P \leq 0.05$) in the combination of 70RES and ANTI than in the other treatments. Tilmicosin phosphate increased the time elapsed from LPS challenge until the maximum IFN-γ concentration was reached in 70AL but decreased the same response in 70RES ($P = 0.05$). For ovalbumin IgG concentration, tilmicosin phosphate tended ($P = 0.08$) to have opposite effects, increasing the concentration in 70RES but decreasing it in 70AL. Explanations for the mode of action of these effects are not readily apparent; however, the humoral response to ovalbumin was numerically ($P = 0.08$) greatest in the treatment that also exhibited the greatest numerical ($P = 0.37$) IL-4 response (70RES + ANTI; Table 5). This finding might be expected, because antiinflammatory cytokines support humoral immunity (Elenkov et al., 2005; Carroll and Forsberg, 2007).

As expected, ovalbumin IgG concentration increased at 12 and again at 18 d after ovalbumin injection ($P < 0.001$; data not shown), but IgG concentrations did not differ among diets ($P = 0.86$; Table 5). In contrast to the current study, Whitney et al. (2006) found that hay-based diets increased IgG concentration after vaccination compared with concentrate-based diets. Dietary treatments might alter both acute (PIC) and longer-term immune responses (IgG) in cattle; however, the relationships seem complex and could potentially interact with other treatments the animals receive.

Other Results

Positive correlations ($P < 0.003$) were found among temperature and cortisol, IL-6, and TNF-α during the

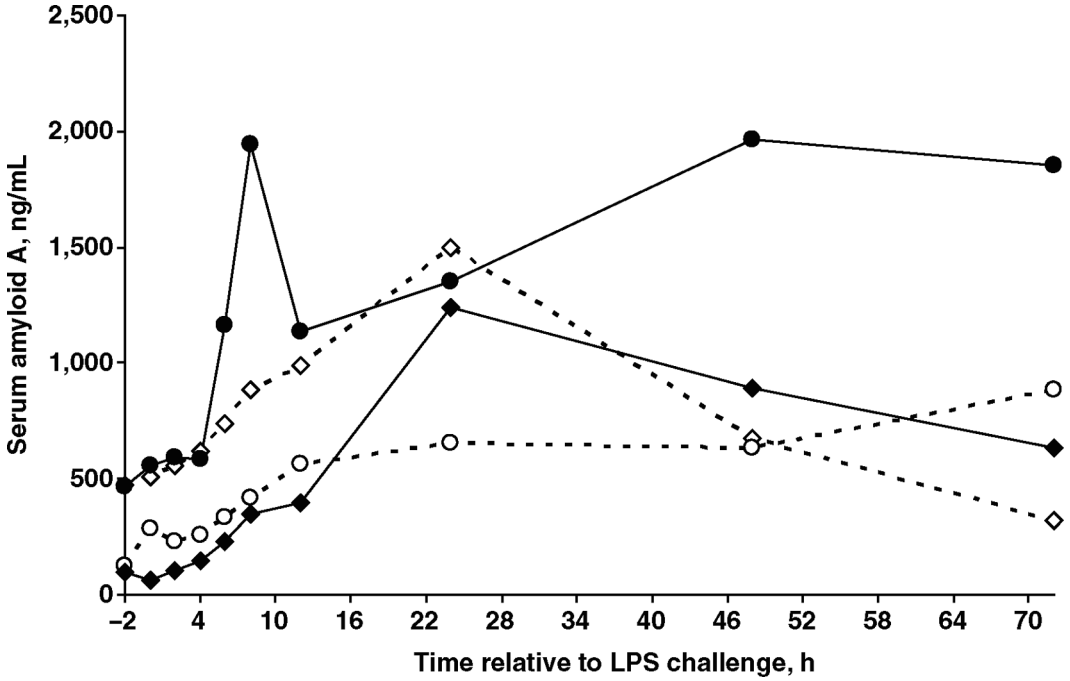

Figure 7. Least squares means of serum amyloid A (SAA) concentration in steers challenged with lipopolysaccharide (LPS; n = 4 steers/treatment; simple effect SE = 331). Dietary treatments were a 70% concentrate diet fed ad libitum (♦), a 30% concentrate diet fed ad libitum (not shown), and a 70% concentrate diet fed in a quantity restricted to equal the NE_g intake of the 30% concentrate treatment (●). Within dietary treatments, steers that were injected with 30 mg of tilmicosin phosphate/kg of BW are represented with dashed lines and open symbols, whereas steers that received saline are represented with solid lines and symbols. An interaction of energy intake × antibiotic treatment was detected for the overall SAA response across time in a repeated-measures model (P = 0.002), in that SAA was decreased by tilmicosin phosphate in the 70% restricted intake treatment but not in the 70% ad libitum treatment.

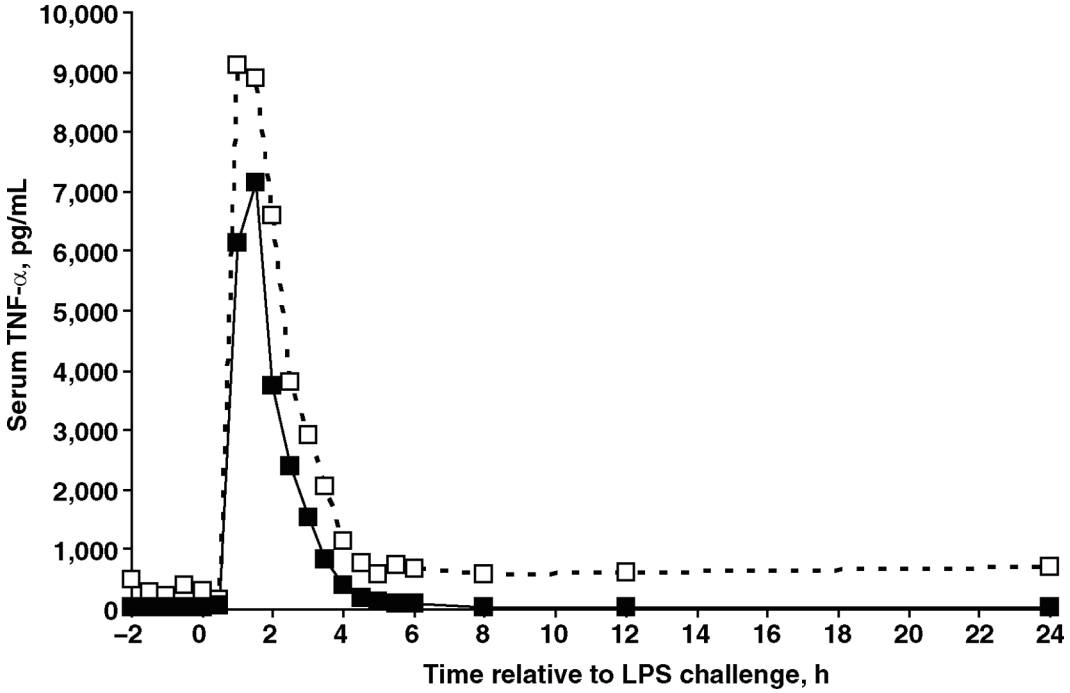

Figure 8. Least squares means of serum tumor necrosis factor-α (TNF-α) in steers challenged with lipopolysaccharide (LPS; largest main effect × sampling time SE = 691). Treatments were injection of 30 mg of tilmicosin phosphate/kg of BW (□, n = 9 to 12) or saline (■, n = 12). A trend was detected for injection of tilmicosin phosphate to increase the overall TNF-α response across time in a repeated-measures model (P = 0.09).

challenge period (−2 to 12 h RTC; r = 0.45, 0.21, and 0.17, respectively). Average daily gain was negatively correlated with IL-6 concentration over the 50-d experimental period (r = −0.54; $P = 0.009$). Previous research in our laboratory detected a similar negative relationship between IL-6 and ADG in beef heifers received in a commercial environment for 44 d (Reuter, 2007).

Ingredients used in receiving diets for beef calves have traditionally been selected on the basis of price and potential effects on performance. Our results indicate that decreasing the diet concentrate:roughage ratio increased production of PIC in response to a LPS challenge in beef steers. A portion of this response seemed to be caused by decreased energy intake, whereas the remainder seemed to be a direct effect of the ingredients themselves (e.g., grain vs. roughage). In addition, administration of tilmicosin phosphate accelerated the febrile response to the LPS challenge. Tilmicosin elevated prechallenge concentration of PIC and decreased SAA AUC after the challenge in cattle on a lower energetic plane of nutrition but not in cattle on a higher plane. Consideration of potential immunomodulating effects of diets, and how those effects might interact with cattle that have already been treated for disease, may be warranted in future studies. Additional research is needed to confirm the present results, to document changes in immune cell populations resulting from these dietary treatments, and to determine the effects of the intensity of PIC response on cattle health in a commercial environment.

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